Claims:

We claim:

1. A composition comprising a protected alkylating reagent wherein deprotection of said reagent is catalyzed by an enzyme.

2. The composition of claim 1, wherein said reagent includes a protecting group selected from the group consisting of a phosphate, an ester, a carbohydrate, a nucleic acid, and a lipid.

3. The reagent of claim 1, wherein said enzyme is selected from the group consisting of glycosidases, nucleases, lipases, esterases, hydroxylases and phosphatases.

4. The reagent of claim 3, wherein said enzyme is a phosphatase.

5. The reagent of claim 3, wherein said enzyme is a glycosidase.

6. The composition of claim 1, wherein said reagent is a 4-halobutadienyl ether or ester.

7. The composition of claim 1, wherein said reagent is a 2-halovinyl ether or ester.

8. The reagent of claim 7, wherein said 2-halovinyl ether or ester is a 2-halovinyl monophosphate.

9. The reagent of claim 7, wherein said vinyl group is substituted with one or two alkyl or aryl groups.

10. The reagent of claim 9, wherein said alkyl or aryl groups are substituted or unsubstituted.

11. The composition of claim 1, wherein said protected alkylating reagent is an α-haloketone.

12. The composition of claim 1, wherein said protected alkylating reagent is α-bromoacetylbenzoic acid (BABA) or α-chloroacetylbenzoic acid (CABA).

13. The composition of claim 1, further comprising a nucleophilic agent.

14. The composition of claim 1, further comprising a disulfide reducing agent.

15. The reagent of claim 14, wherein said disulfide reducing agent is a phosphine.

16. The reagent of claim 15, wherein said phosphine is tris(carboxyethyl)phosphine.

17. A kit for use in carrying out a coupling reaction comprising, in a packaged combination, a first reagent comprising a protected alkylating reagent, in an amount sufficient to conduct at least one reaction.

18. The kit of claim 17, further comprising a second reagent comprising a catalyst capable of deprotecting said protected alkylating reagent.

19. A kit for use in a method for detecting and determining the amount of homocysteine in a sample, comprising in a packaged combination: a first reagent comprising a protected alkylating reagent capable of chemically modifying homocysteine to form modified homocysteine when deprotected, a second reagent comprising an activating reagent capable of deprotecting said protected alkylating reagent, and a third reagent capable of specifically binding to said modified homocysteine, each in an amount sufficient to conduct at least one assay.

20. The kit of claim 19, wherein said first reagent comprises a protected haloketone having a phosphate protecting group.

21. The kit of claim 19, wherein said first reagent further comprises a homocysteine disulfide reducing agent.

22. The kit of claim 19, wherein said first reagent further comprises a solid matrix coated with modified homocysteine.

23. The kit of claim 22, wherein said solid matrix comprises latex or glass beads.

24. The kit of claim 20, wherein said protected haloketone is CABA or BABA.

25. The kit of claim 19, wherein said second reagent further comprises a phosphatase.

26. The kit of claim 25, wherein said phosphatase is alkaline phosphatase.

27. The kit of claim 19, wherein said second reagent further comprises a solid matrix coated with a receptor capable of specifically binding modified homocysteine.

28. The kit of claim 27, wherein said receptor is an antibody or an immunologically active fragment thereof.

29. The kit of claim 22 or 27, wherein said matrix further includes a signaling agent affixed thereto.

30. The kit of claim 29, wherein said signaling agent comprises a chemiluminescent agent, a fluorescent agent, or a chromogenic agent.

31. A method of preparing molecular conjugates, comprising the following steps:

(a) labeling a first molecule with a protected alkylating reagent;

(b) admixing said labeled first molecule with a second molecule, wherein said second molecule contains one or more nucleophilic groups attached thereto; and an enzyme to initiate a coupling reaction.

32. A method of determining the amount of homocysteine in a sample suspected of containing said homocysteine, comprising the steps of:

(a) bringing together in an aqueous medium:

(1) said sample,

(2) a first reagent comprising a protected alkylating reagent capable of being activated to chemically modify the sulfhydryl groups of homocysteine to form modified homocysteine, and

(3) a second reagent comprising a ligand capable of specifically binding to said modified homocysteine to form an immunocomplex; and

(4) a third reagent capable of activating said protected alkylating reagent.

(b) measuring the amount of said immunocomplex, the amount thereof being related to the amount of homocysteine in said sample.

33. The method of claim 32, wherein said first reagent further comprises a disulfide reducing agent.

34. The method of claim 32, wherein said protected alkylating reagent is a halovinyl ether or ester.

35. The method of claim 34, wherein said halovinyl ether or ester is an α-haloketone enol phosphate.

36. The method of claim 35, wherein said α-haloketone enol phosphate is selected from the group consisting of BABA enol phosphate and CABA enol phosphate.

37. The method of claim 32, wherein said third reagent is a phosphatase.

38. The method of claim 37, wherein said phosphatase is alkaline phosphatase.

39. The method of claim 32, wherein said first reagent further comprises a solid matrix coated with hcy-ABA.

40. The method of claim 32, wherein said first reagent further comprises a solid matrix coated with a receptor capable of binding modified homocysteine.

41. The method of claims 39 or 40, wherein said solid matrix comprises latex or glass beads.

42. The method of claims 39 or 40, wherein said solid matrix comprises a microtiter plate.

43. The method of claim 40, wherein said receptor is an antibody or an immunologically active fragment of an antibody.

44. A method of determining the amount of homocysteine in a sample, wherein at least a portion of said homocysteine is in the disulfide form, comprising the steps of:

(a) preparing an admixture comprising:

(1) said sample,

(2) a releasing agent to release said homocysteine from the disulfide form,

(3) a protected alkylating reagent capable of being activated to chemically modify the sulfhydryl groups of homocysteine to form modified homocysteine, and

(4) a receptor capable of specifically binding to said modified homocysteine to form an immunocomplex; and

(5) an activating reagent capable of deprotecting said protected alkylating reagent.

(b) examining said medium for the amount of said immunocomplex, the amount thereof being related to the amount of homocysteine in said sample.

45. A method of preparing a stable, protected haloketone comprising the phosphorylation of said haloketone to form its corresponding enol phosphate.

46. In a method for determining the amount of homocysteine in a sample wherein the homocysteine is modified by a reagent, the improvement comprising providing a precursor to said reagent and an enzyme capable of converting said precursor to said reagent.

47. A method for releasing an alkylating reagent into an aqueous medium comprising combining, in an aqueous solution,

an enol ether of an α-haloketone, an enol ester of an α-haloketone, an enol ether of an γ-halo-α,β-unsaturated ketone or an enol ester of an γ-halo-α,β-unsaturated ketone, and

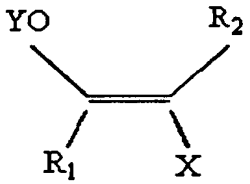
an enzyme capable of hydrolyzing said enol ether or ester.

48. The method of claim 47 wherein said enol ester is an enol phosphate and said enzyme is a phosphatase.

49. The method of claim 47 wherein said aqueous solution further comprises a compound that becomes alkylated subsequent to said combining step.

50. A method of alkylating a mercaptan in an aqueous solution comprising combining said mercaptan with an enol ester of an α-haloketone or the enol ester of an γ-halo-α,β-unsaturated ketone, and
an enzyme capable of hydrolyzing said enol ester.

51. A protected haloketone according to the following formulation:



wherein R_1 and R_2 are alkyl, aryl or substituted alkyl or aryl; X is Cl, Br or I; and Y is a protecting group that may be removed by an enzyme.

52. A protected haloketone according to claim 51, wherein R_1 is $-C_6H_4COOH$, R_2 is H, X is Br and Y is $-PO_3H_2$.

53. A protected haloketone according to claim 51, wherein R_1 is $-C_6H_4CONHZ$, R_2 is H, X is Cl and Y is $-PO_3H_2$.

54. A protected haloketone according to claim 53, wherein Z is H or NH_2.

55. A protected haloketone according to claim 53, wherein Z is selected from the group consisting of proteins, polypeptides, oligonucleotides, polysaccharides, and lipids.

56. A protected haloketone according to claim 52 or 53, wherein said enzyme is alkaline phosphatase.